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(FILE 'HOME' ENTERED AT 17:21:27 ON 26 OCT 2004)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 17:22:45 ON 26 OCT 2004

SEA (EUGENO? OR (FERUL?(S)ACID?))(S)(CONIFERY? OR VANILL?)

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189 FILE AGRICOLA
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1917 FILE USPATFULL
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210 FILE WPIDS
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210 FILE WPINDEX
15 FILE IPA
9 FILE NAPRALERT
8 FILE NLDB

L1 QUE (EUGENO? OR (FERUL?(S) ACID?))(S)(CONIFERY? OR VANILL?)

FILE 'USPATFULL, CAPLUS, BIOSIS, CABA, SCISEARCH, PASCAL, FSTA,
TOXCENTER, ESBIOBASE, IFIPAT, WPIDS, EMBASE, LIFESCI, AGRICOLA, MEDLINE'
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L2 7712 S (EUGENO? OR (FERUL?(S)ACID?))(S)(CONIFERY? OR VANILL?)
L3 210 S L2(S)(DEHYDROGENAS? OR SYNTHAS? OR SYNTHETAS? OR KETOTHIOLAS
L4 37 S L3 (S)(INACTIVA? OR DELET? OR INSERT?)
L5 9 DUP REM L4 (28 DUPLICATES REMOVED)

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=> index bioscience medicine
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
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FULL ESTIMATED COST                                         0.42       0.42

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      CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 17:22:45 ON 26 OCT 2004

78 FILES IN THE FILE LIST IN STNINDEX

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         36   FILE DRUGB
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 61 FILES HAVE ONE OR MORE ANSWERS,   78 FILES SEARCHED IN STNINDEX

L1   QUE (EUGENO? OR (FERUL?(S) ACID?))(S)(CONIFERY? OR VANILL?)
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F2          1612   CAPLUS
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F61            1   SYNTHLINE

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                                                 ENTRY    SESSION
FULL ESTIMATED COST                               2.85       3.27

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                OR DEMETHYLAS?)

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MISSING TERM BEFORE '(S'
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PROCESSING COMPLETED FOR L4
L5            9 DUP REM L4 (28 DUPLICATES REMOVED)

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L5 ANSWER 1 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
TI PRODUCTION OF P-HYDROXYBENZOIC ACID

=> d ti l5 2-9

L5 ANSWER 2 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
TI PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID AND COENZYME A (COASH) UNDER TRANS-FERULATE:COASH LIGASE ENZYME ACTIVITY, TRANS-FERULOYL SCOA HYDRATASE ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS ENZYMES

L5 ANSWER 3 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 1
TI Functional analyses of genes involved in the metabolism of ferulic acid in Pseudomonas putida KT2440

L5 ANSWER 4 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 2
TI Cloning and characterization of the ferulic acid catabolic genes of Sphingomonas paucimobilis SYK-6

L5 ANSWER 5 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
TI PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID AND COENZYME A (COASH) UNDER TRANS-FERULATE:COASH LIGASE ENZYME ACTIVITY, TRANS-FERULOYL SCOA HYDRATASE ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS ENZYMES

L5 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
TI Organisms with inactivated enzymes of eugenol and/or ferulic acid catabolism and their use for production of substituted phenols

L5 ANSWER 7 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 3
TI Bioconversion of ferulic acid into vanillic acid by means of a vanillate-negative mutant of Pseudomonas fluorescens strain BF13

L5 ANSWER 8 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 4
TI Biochemical and genetic analyses of ferulic acid catabolism in Pseudomonas sp strain HR199

L5 ANSWER 9 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 5
TI Biotransformation of eugenol to vanillin by a mutant of Pseudomonas sp strain HR199 constructed by disruption of the vanillin dehydrogenase (vdh) gene

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L5 ANSWER 1 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
AN 10423087 IFIPAT;IFIUDB;IFICDB
TITLE: PRODUCTION OF P-HYDROXYBENZOIC ACID
INVENTOR(S): Gasson; Michael John, Norfolk, GB
Narbad; Arjan, Norfolk, GB
Rhodes; Michael John Charles, Norfolk, GB
Walton; Nicholas John, Norfolk, GB
PATENT ASSIGNEE(S): Unassigned
AGENT: Michael L. Goldman NIXON PEABODY LLP, Clinton Square, P.O. Box 31051, Rochester, NY, 14603-1051, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003167511	A1	20030904
APPLICATION INFORMATION:	US 2002-199405		20020717

APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS

Section 371 PCT Filing OF:	WO 1997-GB809	19970324	UNKNOWN
DIVISION OF:	US 1998-155185	19980922	
DIVISION OF:	US 2000-733383	20001207	

	NUMBER	DATE
PRIORITY APPLN. INFO.:	GB 1996-6187	19960323
FAMILY INFORMATION:	US 2003167511	20030904
DOCUMENT TYPE:	Utility	
	Patent Application - First Publication	
FILE SEGMENT:	CHEMICAL	
	APPLICATION	

NUMBER OF CLAIMS: 86 19 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 describes the **vanillin** pathway in Pseudomonas fluorescens biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-beta-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-**ferulic acid** and transferuloyl SCoA; II is an enzyme that catalyses the interconversion of trans-**feruloyl** SCoA and HMPHP SCOA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and ***vanillin*** ; and IV is an enzyme that catalyses the interconversion of ***vanillin*** and **vanillic acid**.

FIG. 2 illustrates the growth of strain AN103 following transfer to MM medium containing 10 mM **vanillate** (V), 10 mM transferulate (F) or 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV). Cultures were previously grown in MM medium containing 10 mM **vanillate**.

FIG. 3 indicates the changes in trans-**ferulate** and **vanillate** concentrations during growth of strain AN 103 on MM medium containing 10 mM trans-fermlate.

FIG. 4 shows the production of **vanillin** (van) and **vanillate** (VA) by an extract of cells of strain AN103 (165 mu g protein) incubated with trans-**ferulate**, ATP, CoASH and Mg2+ ions, both in the absence of NAD+ and in its presence (0.5 mM). Cells were grown in the presence of 10 mM trans-***ferulate*** , plus 10 mM **vanillate**.

FIG. 5 demonstrates the formation of **feruloyl** SCoA, **vanillin** and acetyl SCoA from trans-**ferulate** supplied to a PD10-treated extract of trans-**ferulate**-grown cells of strain AN103 (7 mu g protein) in the presence of ATP, CoASH and Mg2+ ions.

FIG. 6 demonstrates the production of **vanillin**, acetyl SCoA and ***feruloyl*** SCoA from HMPHP SCoA supplied to a PD10-treated cellfree extract (7 mu g protein) of trans-**ferulate**-grown cells of strain AN103.

FIG. 7 shows the induction over time of trans-**ferulate**:CoASH ligase activity in response to 10 mM trans-**ferulate** (F), 10 mM ***vanillate*** (V) and 10 mM trans-**ferulate** plus 10 mM ***vanillate*** (FV) present in MM medium. The inocula were grown in MM medium plus 10 mM **vanillate**; growth,conditions, enzyme extraction and assay were as described in Examples 1 and 2.

FIG. 8 shows SDS-PAGE of A), an extract of cells grown in MM medium with 10 mM trans-**ferulate**, electrophoresed at successive stages of purification of the HMPHP SCoA cleavage enzyme; successive stages are Crude Extract, Mono Q-purified, Mono-Ppurified and Phenyl Superose-purified, and B), extracts of cells grown in MM medium with either 10 mM **vanillate** or 10 mM trans-***ferulate*** and electrophoresed alongside Mono-P-purified cleavage enzyme; A) silver-stained; B) Coomassie-stained .

FIG. 9 shows EcoRI/PstI digests of cosmid clones pFI793, pFI794, pFI795 and pFI796 separated on an agarose gel.

FIG. 10 shows the sequence of the redundant primers designed from 20 N-terminal amino residues of the 31-kDal protein (SEQ ID Nos. 5 and 6).

FIG. 11 shows a Southern blot of EcoRI/PstI digests of various cosmid clones probed with the PCR product amplified using the Nterminal degenerate oligonucleotide primers as shown in FIG. 10.

FIG. 12 shows the nucleotide sequence of pFI989 (ie the 4370 bp EcoRI/PstI fragment from pFI794), together with the succeeding 882 bp determined from a further subclone, pFI1056 and from pFI794 itself (SEQ ID No 7). The amino ***acid*** sequence of the 31 kD protein and that corresponding to the succeeding open reading frame encoding **vanillin**:NAD+ oxidoreductase (***vanillin*** **dehydrogenase**) (SEQ ID Nos. 2 and 4) are also shown.

FIG. 13 shows the nucleotide sequence of pFI901 (ie the 1.8 kb EcoRI/PstI fragment from pFI793) (SEQ ID No 8).

FIG. 14 shows the nucleotide sequence of pFI911 (ie the 850 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 9).
FIG. 15 shows the nucleotide sequence of pFI912 (ie the 958 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 10).
FIG. 16 shows the nucleotide sequence of pFI913 (ie the 959 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 11).
FIG. 17 is a diagranunatic representation of the outward reading primers for pFI901 (P35 and P39), pFI911 (P32 and P36), pFI912 (P33 and P37) and pFI913 (P34 and P38).
FIG. 18 is a diagrammatic representation showing the formation of the 1.5 kb PCR product, using primers P34 and P39, which spans the region in the cosmid between the **inserts** of pFI913 and pFI901.
FIG. 19 shows the nucleotide sequence of the merged contigs pFI913/PCR product/pFI901 (4259 bp) (SEQ ID No 12).

AB One aspect of the present invention relates to a transgenic plant which, by presence of a transgene, is able to produce phydroxybenzoic acid or a beta -D-glycoside or beta -D-glucose ester thereof. A method is also disclosed for producing phydroxybenzoic acid or a beta -D-glycoside or beta -D-glucose ester thereof using a transgenic plant of the present invention.

CLMN 86 19 Figure(s).

FIG. 1 describes the **vanillin** pathway in Pseudomonas fluorescens biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-beta-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-**ferulic acid** and transferuloyl SCoA; II is an enzyme that catalyses the interconversion of trans-**feruloyl** SCoA and HMPHP SCOA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and **vanillin**; and IV is an enzyme that catalyses the interconversion of **vanillin** and **vanillic acid**.

FIG. 2 illustrates the growth of strain AN103 following transfer to MM medium containing 10 mM **vanillate** (V), 10 mM transferulate (F) or 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV). Cultures were previously grown in MM medium containing 10 mM **vanillate**.

FIG. 3 indicates the changes in trans-**ferulate** and **vanillate** concentrations during growth of strain AN 103 on MM medium containing 10 mM trans-fermlate.

FIG. 4 shows the production of **vanillin** (van) and **vanillate** (VA) by an extract of cells of strain AN103 (165 mu g protein) incubated with trans-**ferulate**, ATP, CoASH and Mg2+ ions, both in the absence of NAD+ and in its presence (0.5 mM). Cells were grown in the presence of 10 mM trans-**ferulate**, plus 10 mM **vanillate**.

FIG. 5 demonstrates the formation of **feruloyl** SCoA, **vanillin** and acetyl SCoA from trans-**ferulate** supplied to a PD10-treated extract of trans-**ferulate**-grown cells of strain AN103 (7 mu g protein) in the presence of ATP, CoASH and Mg2+ ions.

FIG. 6 demonstrates the production of **vanillin**, acetyl SCoA and **feruloyl** SCoA from HMPHP SCoA supplied to a PD10-treated cellfree extract (7 mu g protein) of trans-**ferulate**-grown cells of strain AN103.

FIG. 7 shows the induction over time of trans-**ferulate**:CoASH ligase activity in response to 10 mM trans-**ferulate** (F), 10 mM **vanillate** (V) and 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV) present in MM medium. The inocula were grown in MM medium plus 10 mM **vanillate**; growth,conditions, enzyme extraction and assay were as described in Examples 1 and 2.

FIG. 8 shows SDS-PAGE of A), an extract of cells grown in MM medium with 10 mM trans-**ferulate**, electrophoresed at successive stages of purification of the HMPHP SCoA cleavage enzyme; successive stages are Crude Extract, Mono Q-purified, Mono-Ppurified and Phenyl Superose-purified, and B), extracts of cells grown in MM medium with either 10 mM **vanillate** or 10 mM trans-**ferulate** and electrophoresed alongside Mono-P-purified cleavage enzyme; A) silver-stained; B) Coomassie-stained .

FIG. 9 shows EcoRI/PstI digests of cosmid clones pFI793, pFI794, pFI795 and pFI796 separated on an agarose gel.

FIG. 10 shows the sequence of the redundant primers designed from 20 N-terminal amino residues of the 31-kDal protein (SEQ ID Nos. 5 and 6).

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FIG. 14 shows the nucleotide sequence of pFI911 (ie the 850 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 9).

FIG. 15 shows the nucleotide sequence of pFI912 (ie the 958 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 10).

FIG. 16 shows the nucleotide sequence of pFI913 (ie the 959 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 11).

FIG. 17 is a diagranunatic representation of the outward reading primers for pFI901 (P35 and P39), pFI911 (P32 and P36), pFI912 (P33 and P37) and pFI913 (P34 and P38).

FIG. 18 is a diagrammatic representation showing the formation of the 1.5 kb PCR product, using primers P34 and P39, which spans the region in the cosmid between the **inserts** of pFI913 and pFI901.

FIG. 19 shows the nucleotide sequence of the merged contigs pFI913/PCR product/pFI901 (4259 bp) (SEQ ID No 12).

L5 ANSWER 2 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN

AN 03987942 IFIPAT;IFIUDB;IFICDB

TITLE: PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID AND COENZYME A (COASH) UNDER TRANS-FERULATE:COASH LIGASE ENZYME ACTIVITY, TRANS-FERULOYL SCOA HYDRATASE ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS ENZYMES

INVENTOR(S): Gasson; Michael John, Norfolk, GB
Narbad; Arjan, Norfolk, GB
Rhodes; Michael John Charles, Norfolk, GB
Walton; Nicholas John, Norfolk, GB

PATENT ASSIGNEE(S): Plant Bioscience Limited, Norwich, GB

PRIMARY EXAMINER: Saidha, Tekchand

AGENT: Nixon Peabody LLP

	NUMBER	PK	DATE
PATENT INFORMATION:	US 6664088	B2	20031216
	US 2001014467	A1	20010816
APPLICATION INFORMATION:	US 2000-733383		20001207

EXPIRATION DATE: 3 May 2019

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
DIVISION OF:	US 1999-155183	19990503	6323011

	NUMBER	DATE
PRIORITY APPLN. INFO.:	GB 1996-6187	19960323
FAMILY INFORMATION:	US 6664088	20031216
	US 6323011	
	US 2001014467	20010816

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted Patent - Utility, with Pre-Grant Publication
CHEMICAL
GRANTED

PARENT CASE DATA:

This application is a divisional of U.S. patent application Ser. No. 09/155,183 (now U.S. Pat. No. 6,323,011 B1), which was filed on May 3, 1999 (and accepted May 3, 1999) under 35 U.S.C. section 371 as a national stage application of

PCT/GB97/00809 filed Mar. 24, 1997, claiming priority of Great Britain Application No. 9606187.4 filed Mar. 23, 1996. The biological material listed below has been deposited under the Budapest Treaty at The National Collections of Industrial and Marine Bacteria Limited (23 St. Machar Drive, Aberdeen AB2 1RY, Scotland, UK):

** TABLE **

NCIMB No. Description Date of Deposit 40783 Pseudomonas fluorescens biovar V (strain Jan. 15, 1996 AN103) 40777 Escherichia coli (strain pFI793) containing Dec. 15, 1995 cosmid pFI703

NOTE: INDEXED FROM APPLICATION
NUMBER OF CLAIMS: 16
GRAPHICS INFORMATION: 27 Drawing Sheet(s), 27 Figure(s).
DESCRIPTION OF FIGURES:

FIG. 1 describes the **vanillin** pathway in Pseudomonas fluorescens biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-beta-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-**ferulic acid** and transferuloyl SCoA; II is an enzyme that catalyses the interconversion of trans-**feruloyl** SCoA and HMPHP SCoA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and ***vanillin*** ; and IV is an enzyme that catalyses the interconversion of ***vanillin*** and **vanillic acid**.

FIG. 2 illustrates the growth of strain AN103 following transfer to MM medium containing 10 mM **vanillate** (V), 10 mM transferulate (F) or 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV). Cultures were previously grown in MM medium containing 10 mM **vanillate**.

FIG. 3 indicates the changes in trans-**ferulate** and **vanillate** concentrations during growth of strain AN 103 on MM medium containing 10 mM trans-**ferulate**.

FIG. 4 shows the production of **vanillin** (van) and **vanillate** (VA) by an extract of cells of strain AN103 (165 mu g protein) incubated with trans-**ferulate**, ATP, CoASH and Mg2+ ions, both in the absence of NAD+ and in its presence (0.5 mM). Cells were grown in the presence of 10 mM trans-***ferulate*** , plus 10 mM **vanillate**.

FIG. 5 demonstrates the formation of **feruloyl** SCoA, **vanillin** and acetyl SCoA from trans-**ferulate** supplied to a PD10-treated extract of trans-**ferulate**-grown cells of strain AN 103 (7 kg protein) in the presence of ATP, CoASH and Mg2+ ions.

FIG. 6 demonstrates the production of **vanillin**, acetyl SCoA and ***feruloyl*** SCoA from HMPHP SCoA supplied to a PD10-treated cellfree extract (7 mu g protein) of trans-**ferulate**-grown cells of strain AN103.

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FIG. 10 shows the sequence of the redundant primers designed from 20 N-terminal amino residues of the 31-kDal protein (SEQ ID Nos. 5 and 6).

FIG. 11 shows a Southern blot of EcoRI/PstI digests of various cosmid clones probed with the PCR product amplified using the Nterminal degenerate oligonucleotide primers as shown in FIG. 10.

FIG. 12 shows the nucleotide sequence of pFI989 (ie the 4370 bp EcoRI/Pstl fragment from pFI794), together with the succeeding 882 bp determined from a further subclone, pFI1056 and from pFI794 itself (SEQ ID No 7). The amino ***acid*** sequence of the 31 kD protein and that corresponding to the succeeding open reading frame encoding **vanillin**:NAD+ oxidoreductase (

vanillin **dehydrogenase**) (SEQ ID Nos. 2 and 4) are also shown.
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FIG. 17 is a diagrammatic representation of the outward reading primers for pFI901 (P35 and P39), pFI911 (P32 and P36), pFI912 (P33 and P37) and pFI913 (P34 and P38).
FIG. 18 is a diagrammatic representation showing the formation of the 1.5 kb PCR product, using primers P34 and P39, which spans the region in the cosmid between the **inserts** of pFI913 and pFI901.
FIG. 19 shows the nucleotide sequence of the merged contigs pFI913/PCR product/pFI901 (4259 bp) (SEQ ID No 12).

AB A method of producing vanillin comprising the steps of: (1) providing trans-ferulic acid or a salt thereof; and (2) providing trans-ferulate: CoASH ligase activity (enzyme activity I), trans-feruloyl ScoA hydratase activity (enzyme activity II), and 4-hydroxy-3-methoxyphenyl- beta -hydroxypropionyl SCoA (HMPHP SCoA) cleavage activity (enzyme activity III). Conveniently the enzymes are provided by Pseudomonas fluorescens Fe3 or a mutant or derivative thereof. Polypeptides with enzymes activities II and III and polynucleotides encoding the polypeptides. Use of the polypeptides or the polynucleotides in a method for producing vanillin is also provided.

NTE INDEXED FROM APPLICATION
CLMN 16
GI 27 Drawing Sheet(s), 27 Figure(s).

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L5 ANSWER 3 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 1

ACCESSION NUMBER: 2003:571208 SCISEARCH
THE GENUINE ARTICLE: 695ZY
TITLE: Functional analyses of genes involved in the metabolism of ferulic acid in Pseudomonas putida KT2440
AUTHOR: Plaggenborg R; Overhage J; Steinbuchel A; Priefert H (Reprint)
CORPORATE SOURCE: Univ Munster, Inst Mikrobiol, Corrensstr 3, D-48149 Munster, Germany (Reprint); Univ Munster, Inst Mikrobiol, D-48149 Munster, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (JUN 2003) Vol. 61, No. 5-6, pp. 528-535.
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
ISSN: 0175-7598.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pseudomonas putida KT2440 is a physiologically extremely versatile non-pathogenic bacterium that is applied as a "biosafety strain" in biotechnological processes, as authorized by the USA National Institute of Health. Analysis of the P. putida KT2440 whole-genome sequence revealed the genetic organization of the genes fcs, ech, and vdh, which are essential for **ferulic acid** conversion to **vanillic acid** via **vanillin**. To confirm the physiological function of these structural genes as **feruloyl**-CoA **synthetase** (Fcs), enoyl-CoA hydratase/aldolase (Ech), and **vanillin dehydrogenase** (Vdh), respectively, they Were cloned and expressed in Escherichia coli. Recombinant strains harboring fcs and ech were able to transform **ferulic acid** to **vanillin**. The enzyme activities of Fcs and Vdh were determined in protein extracts of these cells. The essential involvement of fcs, ech and vdh in the catabolism of **ferulic acid** in P. putida

KT2440 was proven by separately **inactivating** each gene by **insertion** of Omega-elements. The corresponding mutant strains KT2440fcsOmegaKm, KT2440echOmegaKm, and KT2440vdhOmegaKm were not able to grow on **ferulic acid**. The potential application of P. putida KT2440 and the mutant strains in biotechnological **vanillin** production process is discussed.

L5 ANSWER 4 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
DUPLICATE 2

ACCESSION NUMBER: 2002:737818 SCISEARCH
THE GENUINE ARTICLE: 588TX
TITLE: Cloning and characterization of the ferulic acid catabolic genes of Sphingomonas paucimobilis SYK-6
AUTHOR: Masai E (Reprint); Harada Y; Peng X
CORPORATE SOURCE: Nagaoka Univ Technol, Dept Bioengn, Nagaoka, Niigata 9402188, Japan (Reprint); Tokyo Univ Agr & Technol, Grad Sch Bioapplicat & Syst Engn, Koganei, Tokyo 1848588, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (SEP 2002) Vol. 68, No. 9, pp. 4416-4424.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Sphingomonas paucimobilis SYK-6 degrades **ferulic acid** to **vanillin**, and it is further metabolized through the protocatechuate 4,5-cleavage pathway. We obtained a TnS mutant of SYK-6, FA2, which was able to grow on **vanillic acid** but not on **ferulic acid**. A cosmid which complemented the growth deficiency of FA2 on **ferulic acid** was isolated. The 5.2-kb BamHI-EcoRI fragment in this cosmid conferred the transformation activity of **ferulic acid** to **vanillin** on Escherichia coli host cells. A sequencing analysis revealed the genes ferB and ferA in this fragment; these genes consist of 852- and 2,127-by open reading frames, respectively. The deduced amino **acid** sequence of ferB showed 40 to 48% identity with that of the **feruloyl**-coenzyme A (CoA) hydratase/lyase genes of Pseudomonas and Amycolatopsis **ferulic acid** degraders. On the other hand, the deduced amino **acid** sequence of ferA showed no significant similarity to the **feruloyl**-CoA **synthetase** genes of other **ferulic acid** degraders. However, the deduced amino **acid** sequence of ferA did show 31% identity with pimeloyl-CoA **synthetase** of Pseudomonas mendocina 35, which has been classified as a new superfamily of acyl-CoA **synthetase** (ADP forming) with succinyl-CoA **synthetase** (L. B. Sanchez, M. Y. Galperin, and M. Muller, J. Biol. Chem. 275: 5794-5803, 2000). On the basis of the enzyme activity of E. coli carrying each of these genes, ferA and ferB were shown to encode a **feruloyl**-CoA **synthetase** and **feruloyl**-CoA hydratase/lyase, respectively. p-coumaric **acid**, caffeic **acid**, and sinapinic **acid** were converted to their corresponding benzaldehyde derivatives by the cell extract containing FerA and FerB, thereby indicating their broad substrate specificities. We found a ferB homolog, ferB2, upstream of a 5-carboxyvanillic **acid** decarboxylase gene (ligW) involved in the degradation of 5,5'-dehydrodivanillic **acid**. The deduced amino **acid** sequence of ferB2 showed 49% identity with ferB, and its gene product showed **feruloyl**-CoA hydratase/lyase activity with a substrate specificity similar to that of FerB. **Insertional inactivation** of each fer gene in S. paucimobilis SYK-6 suggested that the ferA gene is essential and that ferB and ferB2 genes are involved in **ferulic acid** degradation.

L5 ANSWER 5 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
AN 10014465 IFIPAT;IFIUDB;IFICDB
TITLE: PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID AND COENZYME A (COASH) UNDER TRANS-FERULATE:COASH LIGASE ENZYME ACTIVITY, TRANS-FERULOYL SCOA HYDRATASE ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-

HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS ENZYMES

INVENTOR(S): Gasson; Michael John, Norfolk, GB
Narbad; Arjan, Norfolk, GB
Rhodes; Michael John Charles, Norfolk, GB
Walton; Nicholas John, Norfolk, GB

PATENT ASSIGNEE(S): Unassigned

PATENT ASSIGNEE PROBABLE: Plant Bioscience Ltd GB (Probable)

AGENT: Michael L. Goldman NIXON PEABODY LLP, Clinton Square, P.O. Box 31051, Rochester, NY, 14603, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2001014467	A1	20010816
APPLICATION INFORMATION:	US 2000-733383		20001207

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
Section 371 PCT Filing OF:	WO 1997-GB809	19970324	UNKNOWN
DIVISION OF:	US 1999-155183	19990503	

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PRIORITY APPLN. INFO.:	GB 1996-61874	19960323
FAMILY INFORMATION:	US 2001014467	20010816
	US 6664088	20031216

DOCUMENT TYPE: Utility

FILE SEGMENT: Patent Application - First Publication
CHEMICAL
APPLICATION

NUMBER OF CLAIMS: 66 19 Figure(s).

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CLMN 66 19 Figure(s).

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L5 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:289140 CAPLUS
DOCUMENT NUMBER: 132:319715
TITLE: Organisms with inactivated enzymes of eugenol and/or ferulic acid catabolism and their use for production of substituted phenols
INVENTOR(S): Rabenhorst, Juergen; Steinbuechel, Alexander; Priefert, Horst; Overhage, Joerg
PATENT ASSIGNEE(S): Haarmann & Reimer G.M.b.H., Germany
SOURCE: Ger. Offen., 80 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19850242	A1	20000504	DE 1998-19850242	19981031
WO 2000026355	A2	20000511	WO 1999-EP7952	19991020
WO 2000026355	A3	20001109		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,

CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

BR 9914930	A	20010710	BR 1999-14930	19991020
EP 1124947	A2	20010822	EP 1999-953892	19991020
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 761093	B2	20030529	AU 2000-10413	19991020
JP 2003533166	T2	20031111	JP 2000-579727	19991020
PRIORITY APPLN. INFO.:			DE 1998-19850242 A	19981031
			WO 1999-EP7952 W	19991020

AB The invention concerns a transformed and/or a mutagenized uni- or multi-cellular organism, which is characterized by the fact that enzymes of the eugenol and/or ferulic acid catabolism are inactivated such that an accumulation of the intermediate coniferyl alc., coniferyl aldehyde, ferulic acid, vanillin, and/or vanillic acid takes place. Thus, Pseudomonas with inactivating insertions or deletions in the vdh, or vdh and aat, genes were produced and used in prodn. of vanillin, ferulic acid, and coniferyl alc.

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ACCESSION NUMBER: 2000:424499 SCISEARCH
THE GENUINE ARTICLE: 319TP
TITLE: Bioconversion of ferulic acid into vanillic acid by means of a vanillate-negative mutant of Pseudomonas fluorescens strain BF13
AUTHOR: Civolani C; Barghini P; Roncetti A R; Ruzzi M (Reprint); Schiesser A
CORPORATE SOURCE: UNIV TUSCIA, DIPARTIMENTO AGROBIOL & AGROCHIM, VIA C LELLIS BLOCCO B, I-01100 VITERBO, ITALY (Reprint); UNIV TUSCIA, DIPARTIMENTO AGROBIOL & AGROCHIM, I-01100 VITERBO, ITALY
COUNTRY OF AUTHOR: ITALY
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (JUN 2000) Vol. 66, No. 6, pp. 2311-2317.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 35

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB From a **ferulic-acid**-degrading Pseudomonas fluorescens strain (BF13), we have isolated a transposon mutant, which retained the ability to bioconvert **ferulic acid** into **vanillic acid** but lost the ability to further degrade the Latter **acid**. The mutant, BF13-97, was very stable, and therefore it was suitable to be used as a biocatalyst for the preparative synthesis of **vanillic acid** from **ferulic acid**. By use of resting cells we determined the effect on the bioconversion rate of several parameters, such as the addition of nutritional factors, the concentration of the biomass, and the carbon source on which the biomass was grown. The optimal yield of **vanillic acid** was obtained with cells pregrown on M9 medium containing p-coumaric **acid** (0.1% [wt/vol]) as a sole carbon source and yeast extract (0.001% [wt/vol]) as a source of nutritional factors. Under these conditions, 1 mg (wet weight) of biomass produced 0.23 mg of **vanillic acid** per h, The genomic region of BF13-97 flanking the transposon's site of **insertion** was cloned and sequenced revealing two open reading frames of 1,062 (varA) and 954 (vanB) bp, respectively. The van genes are organized in a cluster and encode the subunits of the **vanillate-O-demethylase** , which catalyzes the first step of the **vanillate** catabolism, Amino **acid** sequences deduced from vanA and vanB genes were shown

to have high identity with known VanAs and VanBs from Pseudomonas and Acinetobacter spp, Highly conserved regions known to exist in class IA oxygenases were also found in the **vanillate**-O-**demethylase** components from P.fluorescens BF13, The terminal oxygenase VanA is characterized by a conserved Rieske-type [2Fe-2S](R) ligand center, The reductase VanB contains a plant-type ferredoxin [2Fe-2S](Fd), flavin mononucleotide, and NAD-ribose binding domains which are located in its C-terminal and N-terminal halves, respectively. Transfer of wild-type vanAB genes to BF13-97 complemented this mutant, which recovered its ability to grow on either **vanillic** or **ferulic acid**.

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ACCESSION NUMBER: 1999:854619 SCISEARCH
THE GENUINE ARTICLE: 252AQ
TITLE: Biochemical and genetic analyses of ferulic acid catabolism in Pseudomonas sp strain HR199
AUTHOR: Overhage J; Priefert H (Reprint); Steinbuchel A
CORPORATE SOURCE: UNIV MUNSTER, INST MIKROBIOL, CORRENSSTR 3, D-48149 MUNSTER, GERMANY (Reprint); UNIV MUNSTER, INST MIKROBIOL, D-48149 MUNSTER, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (NOV 1999) Vol. 65, No. 11, pp. 4837-4847.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The gene loci fcs, encoding **feruloyl** coenzyme A (**feruloyl**-CoA) **synthetase**, ech, encoding enoyl-CoA hydratase/aldolase, and aat, encoding beta-**ketothiolase**, which are involved in the catabolism of **ferulic acid** and **eugenol** in Pseudomonas sp, strain HR199 (DSM7063), were localized on a DNA region covered by two EcoRI fragments (E230 and E94), which were recently cloned from a Pseudomonas sp, strain HR199 genomic library in the cosmid pVK100, The nucleotide sequences of parts of fragments E230 and E94 were determined, revealing the arrangement of the aforementioned genes. To confirm the function of the structural genes fcs and ech, they were cloned and expressed in Escherichia coli, Recombinant strains harboring both genes were able to transform **ferulic acid** to **vanillin**, The **feruloyl**-CoA **synthetase** and enoyl-CoA hydratase/aldolase activities of the fcs and ech gene products, respectively, were confirmed by photometric assays and by high-pressure liquid chromatography analysis. To prove the essential involvement of the fcs, ech, and aat genes in the catabolism of **ferulic acid** and **eugenol** in Pseudomonas sp, strain HR199, these genes were **inactivated** separately by the **insertion** of omega elements. The corresponding mutants Pseudomonas sp, strain HRfcs Omega Gm and Pseudomonas so, strain HRech Omega Km were not able to grow on **ferulic acid** or on **eugenol**, whereas the mutant Pseudomonas sp, strain HRaat Omega Km exhibited a **ferulic acid**- and **eugenol**-positive phenotype like the wild type, In conclusion, the degradation pathway of **eugenol** via **ferulic acid** and the necessity of the activation of **ferulic acid** to the corresponding CoA ester was confirmed. The aat gene product was shown not to be involved in this catabolism, thus excluding a beta-oxidation analogous degradation pathway for **ferulic acid**. Moreover, the function of the ech gene product as an enoyl-CoA hydratase/aldolase suggests that **ferulic acid** degradation in Pseudomonas sp. strain HR199 proceeds via a similar pathway to that recently described for Pseudomonas fluorescens AN103.

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ACCESSION NUMBER: 1999:943195 SCISEARCH

THE GENUINE ARTICLE: 261KJ
TITLE: Biotransformation of eugenol to vanillin by a mutant of Pseudomonas sp strain HR199 constructed by disruption of the vanillin dehydrogenase (vdh) gene
AUTHOR: Overhage J; Priefert H (Reprint); Rabenhorst J; Steinbuchel A
CORPORATE SOURCE: UNIV MUNSTER, INST MIKROBIOL, D-48149 MUNSTER, GERMANY (Reprint); UNIV MUNSTER, INST MIKROBIOL, D-48149 MUNSTER, GERMANY; HAARMANN & REIMER GMBH, D-37601 HOLZMINDEN, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (NOV 1999) Vol. 52, No. 6, pp. 820-828.
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
ISSN: 0175-7598.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The catabolism of **eugenol** in Pseudomonas sp. strain HR199 (DSM7063) proceeds via **coniferyl** alcohol **coniferyl** aldehyde, **ferulic acid**, **vanillin**, **vanillate** and protocatechuate, which is further degraded by the ortho-cleavage pathway. The **vanillin dehydrogenase** of Pseudomonas sp. strain HR199, which catalyses the NAD (+) dependent oxidation of **vanillin** to **vanillate**, was **inactivated** by the **insertion** of omega elements into the vdh gene, which was characterized recently. Omega elements conferring resistance against kanamycin (Omega Km) or gentamycin (Omega Gm) were constructed by polymerase chain reaction amplification of the aminoglycoside 3'-O-phosphotransferase gene and the gentamycin-3-acetyltransferase gene, using the plasmids pSUP5011 and pBBR1MCS-5 respectively as template DNA. A 211-bp BssHII fragment of the vdh gene was substituted by Omega Km or nGm, and the functional vdh gene was replaced by vdh Omega Km or vdh Omega Gm in Pseudomonas sp. strain HR199 by homologous recombination. Cells of the mutant Pseudomonas sp, strain HRvdh Omega Km, pregrown on gluconate, accumulated up to 2.9 mM **vanillin** during incubation in mineral medium with 6.5 mM **eugenol**. As a result of another **vanillin dehydrogenase** activity (VDH-II), the accumulated **vanillin** was further degraded, when **coniferyl** aldehyde was exhausted from the medium. Characterization of the purified VDH-II revealed the identity of this enzyme with the recently characterized **coniferyl**-aldehyde **dehydrogenase**
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INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 17:22:45 ON 26 OCT 2004
SEA (EUGENO? OR (FERUL?(S)ACID?))(S)(CONIFERY? OR VANILL?)

189	FILE AGRICOLA
90	FILE ANABSTR
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                63   FILE JICST-EPLUS
                 8   FILE KOSMET
               192   FILE LIFESCI
               184   FILE MEDLINE
                 6   FILE NIOSHTIC
                 9   FILE NTIS
                 3   FILE OCEAN
               347   FILE PASCAL
                 1   FILE PHIN
               101   FILE PROMT
                 1   FILE RDISCLOSURE
               489   FILE SCISEARCH
                 1   FILE SYNTHLINE
               320   FILE TOXCENTER
              1917   FILE USPATFULL
               144   FILE USPAT2
                 3   FILE VETU
                18   FILE WATER
               210   FILE WPIDS
                 3   FILE WPIFV
               210   FILE WPINDEX
                15   FILE IPA
                 9   FILE NAPRALERT
                 8   FILE NLDB
L1                   QUE (EUGENO? OR (FERUL?(S) ACID?))(S)(CONIFERY? OR VANILL?)
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     TOXCENTER, ESBIOBASE, IFIPAT, WPIDS, EMBASE, LIFESCI, AGRICOLA, MEDLINE'
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L2          7712 S (EUGENO? OR (FERUL?(S)ACID?))(S)(CONIFERY? OR VANILL?)
L3           210 S L2(S)(DEHYDROGENAS? OR SYNTHAS? OR SYNTHETAS? OR KETOTHIOLAS
L4            37 S L3 (S)(INACTIVA? OR DELET? OR INSERT?)
L5             9 DUP REM L4 (28 DUPLICATES REMOVED)

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COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
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